510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
k041349

B. Purpose for Submission:
New device

C. Analyte:
Estradiol

D. Type of Test:
Quantitative

E. Applicant:
IBL-Hamburg

F. Proprietary and Established Names:
IBL Estradiol LIA

G. Regulatory Information:

1. Regulation section:
   21 CFR 862.1260
2. Classification:
   I (reserved per 21 CFR 862.9(a) for new intended use)
3. Product Code:
   CHP
4. Panel:
   75

H. Intended Use:

1. Intended use(s):
   Luminescence immunoassay for the *in vitro diagnostic* quantitative measurement of active free estradiol, an estrogenic steroid, in saliva and serum. Measurements obtained by this device may be used in the diagnosis and treatment of various hormonal sexual disorders and can be used to evaluate ovarian function. This test is not intended for assessing placental function in complicated pregnancy.
2. Indication(s) for use:
   Luminescence immunoassay for the *in vitro diagnostic* quantitative measurement of active free estradiol, an estrogenic steroid, in saliva and serum. Measurements obtained by this device may be used in the diagnosis and treatment of various hormonal sexual disorders and can be used to

evaluate ovarian function. This test is not intended for assessing placental function in complicated pregnancy.

3. Special condition for use statement(s):
   None
4. Special instrument Requirements:
   None

I. Device Description:

The IBL Estradiol LIA kit contains the following:

•Microtiter Plate coated with rabbit anti-mouse antibody
•Estradiol Antiserum (mouse anti-progesterone antibody)
•Standards A-G (0, 0.9, 2, 4, 8, 16, and 64 pg/mL); 17β-estradiol in buffer with BSA and stabilizers
•Controls Level I and II
•Assay Buffer (Tris buffer with BSA and stabilizers)
•Enzyme Conjugate (alkaline phosphatase [calf] conjugate with stabilizers)
•Chemiluminescence Reagent AP (acridan based substrate)
•Wash Buffer (Tris buffer with Tween and stabilizer)
•Adhesive Foil

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Diagnostic Systems Laboratories DSL 4800 Ultra-sensitive Estradiol RIA
2. Predicate K number(s):
   K953605
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicates**
Intended Use	Quantitative measurement of progesterone	Same
Methodology	Immunoassay/antigen competitive binding principle	Same
Differences		
Item	**Device**	**Predicate**
Specimen	Saliva and serum	Serum and plasma
Reading Indicator	Luminescent labeling	Radio labeling

K. Standard/Guidance Document Referenced (if applicable):

None referenced

L. Test Principle:

The IBL Estradiol luminescence immunoassay (LIA) is based on antigen competitive binding. An unknown amount of antigen present in the sample and a fixed amount of enzyme labeled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the reaction. After addition of the luminescence substrate solution the intensity of the luminescence measured is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Three serum samples were diluted ten fold and run in replicates of ten (10) each within one assay run to determine within-run precision. Saliva samples from six (6) individuals were run in replicates of ten (10) each within one assay run to determine within-run precision. The mean concentrations for diluted serum samples ranged from 3.8 to 33.4 pg/mL. The standard deviations (SDs) ranged from 0.3 to 1.0, resulting in %CVs of ≤7.4. The mean concentrations for saliva ranged from 3.5 to 33.0 pg/mL. The standard deviations (SDs) for saliva ranged from 0.3 to 1.2, resulting in %CVs of ≤7.9.

Between run reproducibility was determined by replicate measurements of nine (9) different saliva samples and three (3) different diluted serum samples in ten (10) test runs on ten (10) different days. The mean concentrations for saliva ranged from 1.4 to 54.2 pg/mL. The SDs for saliva ranged from 0.2 to 2.5, resulting in %CVs between 16.5 and 4.6. The mean concentrations for serum ranged from 3.4 to 32.9 pg/mL. The SDs for serum ranged from 0.3 to 1.4, resulting in %CVs between 9.3 and 4.3.

b. Linearity/assay reportable range:

Four (4) commercially available serum samples were initially diluted 1:10 then serially diluted with Standard A from the kit and run in the assay. The measured serum concentrations (prior to the serial dilutions) were 43.3, 30.9, 17.8, and 3.8 pg/mL. All recoveries were within the acceptable range (80-120%) with the exception of two results (one dilution in each of two samples: 74% and 123%).

Three (3) saliva samples were serially diluted two-fold with Standard A from the kit and run in the assay. The measured saliva concentrations prior to dilution were 8.0, 15.5, and 49.6 pg/mL. The recoveries ranged between 80 and 114%.

Three (3) serum and three (3) saliva samples were used to evaluate recovery in the LIA test. The initial serum concentrations were 5.2,

22.1, and 36.4 pg/mL. The initial saliva concentrations were 2.4, 5.5, and 14.6 pg/mL. Purified estradiol was weighed and added at different concentrations to the specimens. Recoveries for the serum samples ranged between 80% and 101%. Recoveries for the saliva samples ranged between 83% and 120%, with the exception of two (122% and 128%), each from a different sample.

c. *Traceability (controls, calibrators, or method):*
Purified estradiol stock

d. *Detection limit:*
The zero Standard A was dispensed in 20 replicates and assayed to assess the lowest level detectable in the assay. The analytical sensitivity (mean minus 2SD) for saliva was 0.3 pg/mL. Steroid free serum (diluted 1:10) was also dispensed in 20 replicates and assayed to assess the lowest level detectable in the assay. The analytical sensitivity (mean minus 2SD) for serum was 0.7 pg/mL (or 7 pg/mL calculated).

Functional sensitivity, the lowest concentration reproducibly measured with a CV <20%, was also determined. A low negative male saliva sample was added to 10 wells and assayed. The mean was 0.8 pg/mL with an SD of 0.15 pg/mL and CV of 19.8%. Two low serum samples were diluted 1:10 and measured 10 times. The mean was 0.8 pg/mL with an SD of 0.1 pg/mL and CV of 16.4%. The calculated functional sensitivity (multiplied by 10) is 8 pg/mL.

e. *Analytical specificity:*
The cross-reactivity of the estradiol antiserum was measured against various compounds. The percent cross-reactivity is expressed as the ratios of estradiol concentration to the concentration of the reacting compound at 50% binding of the zero standard. The percent cross-reactivities were as follows:

17β-Estradiol	100.0
Estrone	0.222
Estriol	0.138
Corticosterone	0.007
Dexamethasone	0.009
Cortisone	0.007
Progesterone	0.012
Testosterone	0.015
Prednisolone	0.005

Cross-reactivities of other substances tested were ≤0.1%.

Interference of triglycerides, bilirubin, and hemoglobin in serum was evaluated. Different concentrations of triglycerides were spiked into two different serum samples. The results demonstrated that high concentrations of up to 30 mg/mL have no effect on measurement. Increasing concentrations of bilirubin were added to three serum samples and assayed. The results demonstrated that bilirubin concentrations up to 0.5 mg/mL do not affect measurement (recoveries ranged from 84-115%). Different concentrations of hemoglobin were spiked into three different serum samples. No significant effect on the results was observed with hemoglobin concentrations up to 1 mg/mL.

The influence of blood, thimerosal, and sodium azide on the assay was also evaluated. A female saliva sample was enriched with different concentrations of whole blood to see the effect on measured estradiol values. The results found that bleeding does not affect results until visible (e.g., >0.25% blood contamination in saliva samples).

Increasing concentrations of thimerosal were added to two saliva samples with known concentrations of estradiol to assess the effect of adding the preservative to saliva on the results. Concentrations of thimerosal up to 0.2% have no significant influence on the results.

Increasing concentrations of sodium azide were added to two saliva samples with known concentrations of estradiol. Concentrations of sodium azide up to 1.0% have no significant influence on the results.

f. Assay cut-off:
See "Detection limit."

2. Comparison studies:

a. Method comparison with predicate device:
The IBL Estradiol LIA was compared to the predicate RIA. Serum samples from healthy adults were assessed by both methods. The tests were performed according to the manufacturer's instructions. Serum samples were diluted 1:10 with Standard A for the IBL LIA. The regression analysis on a total of sixty (60) samples was as follows: $y = 1.00x - 4.83$, $r^2 = 0.95$. The range of samples on the subject device (calculated x10) was 2-444 pg/mL. The range of samples on the predicate device was 22-448 pg/mL.
See "Other clinical supportive data" for information on saliva.

b. Matrix comparison:
A study was performed with the IBL Estradiol LIA to assess the levels of estradiol in serum versus those found in saliva. Saliva and

serum pairs were collected at the same time between 10 a.m. and 4 p.m. then run in the subject device. The results of the comparison yielded the following regression equation: serum = 43.491(saliva) – 4.680, $r^2 = 0.712$.

3. Clinical studies:
   a. *Clinical sensitivity:*
      Not applicable

   b. *Clinical specificity:*
      Not applicable

   c. *Other clinical supportive data (when a and b are not applicable):*
      The IBL Estradiol LIA was compared to a published RIA procedure. The estradiol content from saliva samples collected from apparently healthy females was assessed by both methods. All samples were stored frozen at -20 °C then thawed and assayed. The regression analysis on a total of fifty (50) samples was as follows: $y = 0.96x + 0.55$, $r^2 = 0.97$. The range of samples on the subject device was 0–55 pg/mL. The range of samples on the RIA procedure was 0-51 pg/mL.

4. Clinical cut-off:
   Not applicable

5. Expected values/Reference range:
   A study was conducted to determine a normal range for estradiol levels in saliva. Saliva samples were collected from twenty-eight apparently healthy premenopausal women who were not using contraceptives. Three saliva samples were collected per day (morning, midday, and afternoon), pooled, and frozen prior to running the assay. Collection began at the last day of bleeding and continued daily until the first day of bleeding. In addition, five postmenopausal women and forty males were evaluated. One saliva sample was obtained for these two populations. The range for premenopausal women age 19-43 was found to be 0.6-10.4 pg/mL (follicular phase), 4.5-21.2 pg/mL (mid-cycle peak), and 0.5-10.8 pg/mL (luteal phase). The range for postmenopausal women age 42-62 was found to be <3.2 pg/mL. The range for males age 20-63 was found to be <3.4 pg/mL.

   Note the following mean estradiol levels that have been reported in literature: 2.44 pg/mL (follicular), 4.07 pg/mL (periovulatory), 3.17 pg/mL (luteal), 0.82 pg/mL (non-menstruating girls), and 1.02 pg/mL adult males.

N. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.